

REMARKS

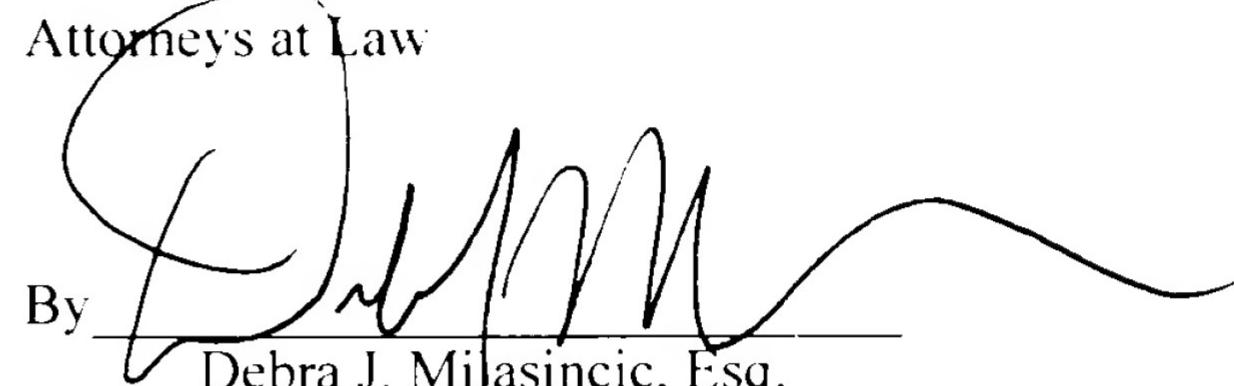
Applicants have attached herewith APPENDIX A setting forth the claims that will be pending after entry of the instant amendment. No new matter has been added to the application.

Date: September 21, 2000

LAHIVE & COCKFIELD, LLP

Attorneys at Law

By



Debra J. Milasincic, Esq.

Reg. No. 46,931

28 State Street

Boston, MA 02109

(617) 227-7400

(617) 742-4214

APPENDIX A

1. A method of producing a panto-compound comprising culturing a microorganism which overexpresses at least one *Bacillus* pantothenate biosynthetic enzyme under conditions such that the panto-compound is produced.
2. The method of claim 1, wherein the microorganism overexpresses at least one *Bacillus subtilis* pantothenate biosynthetic enzyme.
7. A method of producing a panto-compound comprising culturing a ketopantoate reductase-overexpressing (KPAR-O) microorganism under conditions such that the panto-compound is produced.
12. The method of claim 7, wherein the KPAR-O microorganism further overexpresses at least one pantothenate biosynthetic enzyme in addition to overexpressing ketopantoate reductase.
14. A method of producing pantothenate in a manner independent of precursor feed comprising culturing an aspartate- α -decarboxylase-overexpressing (A α D-O) microorganism having a deregulated isoleucine-valine (*ilv*) pathway under conditions such that pantothenate is produced.
15. A method of producing at least 2 g/L pantothenate in a manner independent of aspartate or β -alanine feed comprising culturing an aspartate- α -decarboxylase-overexpressing (A α D-O) microorganism under conditions such that pantothenate is produced.
16. A method of producing at least 2 g/L pantothenate in a manner independent of valine or α -ketoisovalerate feed comprising culturing a microorganism having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway under conditions such that pantothenate is produced.
17. A method of producing at least 30 g/L pantothenate in a manner independent of aspartate or β -alanine feed comprising culturing an aspartate- α -

decarboxylase-overexpressing (AαD-O) microorganism under conditions such that pantothenate is produced.

18. A method of producing at least 30 g/L pantothenate in a manner independent of valine or α-ketoisovalerate feed comprising culturing a microorganism having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway under conditions such that pantothenate is produced.

19. A β-alanine independent high yield production method for producing pantothenate comprising culturing a manipulated microorganism under conditions such that pantothenate is produced at a significantly high yield.

20. The method of claim 14 or 19, wherein the microorganism overexpresses acetohydroxyacid synthetase or is transformed with a vector comprising an *ilvBN* nucleic acid sequence or an *alsS* sequence.

21. The method of claim 14 or 19, wherein the microorganism overexpresses acetohydroxyacid isomeroeductase or is transformed with a vector comprising an *ilvC* nucleic acid sequence.

22. The method of claim 14 or 19, wherein the microorganism overexpresses dihydroxyacid dehydratase or is transformed with a vector comprising an *ilvD* nucleic acid sequence.

23. The method of claim 19, wherein the microorganism overexpresses aspartate-α-decarboxylase or is transformed with a vector comprising a *panD* nucleic acid sequence.

24. The method of claim 14 or 19, wherein the microorganism further has a deregulated pantothenate biosynthetic pathway.

25. The method of claim 14 or 19, wherein the microorganism further has at least one mutant gene selected from the group consisting of a mutant *avtA* gene, a mutant *ilvE* gene, a mutant *ansB* gene and a mutant *alsD* gene.

26. The method of claim 24, wherein the microorganism overexpresses any of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate- α -decarboxylase.

27. The method of claim 24, wherein the microorganism is transformed with a vector comprising a *panBCD* nucleic acid sequence or a vector comprising a *panE1* nucleic acid sequence.

28. The method of claim 14 or 19, wherein pantothenate is produced at a level selected from the group consisting of a level greater than 10g/L, a level greater than 20g/L and a level greater than 40g/L.

29. The method of claim 20, wherein the microorganism overexpresses acetohydroxyacid synthetase derived from *Bacillus* or is transformed with a vector comprising an *ilvBN* nucleic acid sequence or an *alsS* nucleic acid sequence derived from *Bacillus*.

30. The method of claim 21, wherein the microorganism overexpresses acetohydroxyacid isomeroreductase derived from *Bacillus* or is transformed with a vector comprising an *ilvC* nucleic acid sequence derived from *Bacillus*.

31. The method of claim 22, wherein the microorganism overexpresses dihydroxyacid dehydratase derived from *Bacillus* or is transformed with a vector comprising an *ilvD* nucleic acid sequence derived from *Bacillus*.

32. The method of claim 23, wherein the microorganism overexpresses aspartate- α -decarboxylase derived from *Bacillus* or is transformed with a vector comprising a *panD* nucleic acid sequence derived from *Bacillus*.

33. The method of claim 24 [or 26], wherein the microorganism overexpresses any of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate- α -decarboxylase derived from *Bacillus*.

34. The method of claim 27, wherein the vector comprises a *panBC'D* nucleic acid sequence or a *panE1* nucleic acid sequence derived from *Bacillus*.

35. A method of producing a panto-compound comprising contacting a composition comprising at least one pantothenate biosynthesis pathway precursor or isoleucine-valine biosynthesis pathway precursor with at least one isolated *Bacillus* enzyme selected from the group consisting of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate- α -decarboxylase, under conditions such that the panto-compound is produced.

36. A method of producing β -alanine comprising culturing an aspartate- α -decarboxylase-overexpressing (A α D-O) microorganism under conditions such that β -alanine is produced.

37. The method of claim 36, wherein the A α D-O microorganism has a mutation in a nucleic acid sequence encoding a pantothenate biosynthetic enzyme selected from the group consisting of ketopantoate hydroxymethyltransferase, ketopantoate reductase and pantothenate synthetase.

38. A method of producing β -alanine comprising contacting a composition comprising aspartate with an isolated *Bacillus* aspartate- α -decarboxylase enzyme under conditions such that β -alanine is produced.

39. A method for enhancing production of a panto-compound comprising culturing a mutant microorganism having a mutant *coaX* gene under conditions such that the panto-compound production is enhanced.

40. The method of claim 39, wherein said recombinant microorganism has a mutant *coa4* gene.

41. A method of producing a panto-compound comprising a pantothenate kinase mutant microorganism under conditions such that the panto-compound is produced at a significantly high yield.

42. The method of claim 41, wherein said mutant microorganism has a mutant *coaA* gene.

43. The method of claim 41, wherein said mutant microorganism has a mutant *coaX* gene.

44. The method of claim 41, where said mutant microorganism has a mutant *coaA* and *coaX* gene.

46. The method of claim 39 or 41, wherein said panto-compound is pantothenate.

47. The method of claim 39 or 41, wherein said panto-compound is produced at a level selected from the group consisting of a level greater than 10g/L, a level greater than 20g/L and a level greater than 40g/L.

48. The method of claim 39 or 41, wherein said recombinant microorganism further has a deregulated pantothenate biosynthetic pathway or further has a deregulated isoleucine-valine (*ilv*) biosynthetic pathway.

49. The method of claim 39 or 41, wherein said recombinant microorganism further overexpresses *panD* and *panE*.

50. The method of claim 39 or 41, wherein said recombinant microorganism further has at least one mutant gene selected from the group consisting of a mutant *avtA* gene, a mutant *ilvE* gene, a mutant *ansB* gene and a mutant *alsD* gene.

51. A method for enhancing production of a panto-compound comprising culturing a microorganism that has a deregulated pantothenate biosynthetic pathway and that also has a mutation that results in reduced pantothenate kinase activity under conditions such that the panto-compound production is enhanced.

52. A method for identifying compounds which modulate pantothenate kinase activity comprising contacting a recombinant cell expressing pantothenate kinase

encoded by the *coaX* gene with a test compound and determining the ability of the test compound to modulate pantothenate kinase activity in said cell.

53. The method of claim 52, wherein said cell further comprises a mutant *coaA* gene encoding a pantothenate kinase having reduced activity.

62. A recombinant microorganism which overexpresses at least one *Bacillus* pantothenate biosynthetic enzyme.

67. A recombinant microorganism having a mutant *coaX* gene, said mutant *coaX* gene encoding reduced pantothenate kinase activity in said microorganism.

68. The recombinant microorganism of claim 67 further having a mutant *coaA* gene, said mutant *coaA* gene encoding reduced pantothenate kinase activity in said microorganism.

69. A recombinant microorganism having a mutant *coaX* gene and optionally having a mutant *coaA* gene, said mutant microorganism having reduced pantothenate kinase activity as compared to a microorganism having wild-type *coaA* and *coaX* genes.

70. A recombinant microorganism comprising a vector comprising an isolated *coaX* gene.

71. A recombinant microorganism that overproduces a panto-compound, the microorganism having a deregulated pantothenate biosynthetic pathway and having at least one mutation that results in a decrease in the capacity of the microorganism to synthesize Coenzyme A (CoA).

72. The recombinant microorganism of claim 71, having at least one mutation that results in a reduced level of pantothenate kinase activity.

73. The recombinant microorganism of claim 72, having a mutation in a *coaA* gene, or homologue thereof, that results in a reduced level of CoA enzyme activity.

74. The recombinant microorganism of claim 72, having a mutation in a *coaX* gene, or homologue thereof, that results in a reduced level of CoaX enzyme activity.

75. The recombinant microorganism of claim 72, having a mutation in a *coaA* gene, or homologue thereof, and having a mutation in a *coaX* gene, or homologue thereof, the mutations resulting in reduced levels of CoaA enzyme activity and reduced CoaX enzyme activity.

82. A recombinant microorganism selected from the group consisting of PA221, PA235, PA236, PA313, PA410, PA402, PA403, PA411, PA412, PA413, PA303, PA327, PA328, PA401, PA340, PA342, PA404, PA405, PA374, PA354, PA365, PA377, PA651 and PA824.

83. A recombinant vector for use in the production of panto-compounds comprising a nucleic acid sequence which encodes at least one *Bacillus* pantothenate biosynthetic enzyme operably linked to regulatory sequences.

88. A vector comprising a mutant *coaX* gene, said mutant encoding a pantothenate kinase enzyme having reduced activity.

90. A vector comprising an isolated *Bacillus coaX* gene.

97. A vector selected from the group consisting of pAN004, pAN005, pAN006, pAN236, pAN423, pAN428, pAN429, pAN441, pAN442, pAN443, pAN251, pAN267, pAN256, pAN257, pAN263, pAN240, pAN294, pAN296, pAN336, pAN341 and pAN342.

99. An isolated nucleic acid molecule which encodes at least one *Bacillus* pantothenate biosynthetic gene.

102. An isolated *Bacillus* pantothenate biosynthetic enzyme polypeptide.

104. An isolated *Bacillus* ketopantoate reductase polypeptide.

106. An isolated *Bacillus* aspartate- α -decarboxylase polypeptide.
108. An isolated nucleic acid molecule comprising a mutant *coaX* gene.
109. An isolated nucleic acid molecule comprising a *coaX* gene.
110. An isolated pantothenate kinase protein encoded by a *coaX* gene.